

Atty Dkt. No.: STAN-107 DIV
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In the Specification:

Replace the paragraph at Page 3, lines 21 to 25, with:

Newer methods for adenoviral preparation are based on homologous recombination of two plasmids using yeast artificial chromosomes or bacteria. These methods, while more efficient, are more complex. The YAC system requires yeast culture and manipulation while the E.coli system requires three step transformations using an additional non-conventional host bacterial strain (BJ5183recBCsbcBC).

Please replace Page 5, lines 1 -8 with:

three different, non-adenoviral restriction endonuclease sites located in the E region. The second vector is a shuttle vector and includes a non adenoviral nucleic acid (which is desired to be inserted into the adenoviral genome, i.e. an insertion nucleic acid) flanked by two of the three different non-adenoviral endonuclease sites present in the first vector. Cleavage products are prepared from the first and second vectors using the appropriate restriction endonucleases. The resultant cleavage products are then ligated to produce the subject recombinant adenovirus genome. The subject adenoviral genomes find use in a variety of applications, including as vectors for use in a variety of applications, e.g. gene therapy.

Please replace Page 5, lines 11-20 with:

Fig. 1 Construction of recombinant adenovirus vectors by a simple in vitro ligation method. Fig. 1A provides a representation of vector plasmids, pAdHM1, 2, 3 and 4. Fig. 1B provides a representation of shuttle plasmid, pHM3. Fig. 1C provides a scheme for the construction strategy of E1 and E3 deleted adenovirus vector. The expression cassette of interest (RSVhAATbPA) was inserted into the Sall site of the multi-cloning site of pHM3, and the resulting plasmid, pHM3-hAAT1, was digested with I-CeuI and PI-SceI. The fragment containing the hAAT expression cassette was ligated with

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pAdHM4 digested with I-CeuI and PI-SceI. Transformation into DH5 α was performed after the ligation samples were digested with SmaI to reduce the colony formation containing parental vector plasmid (pAdHM4).

Please replace Page 5, lines 23 to 29 with:

In vitro methods for making a recombinant adenovirus genome, as well as kits for practicing the same and the recombinant adenovirus vectors produced thereby, are provided. In the subject methods, the subject genomes are prepared from first and second vectors. The first vector includes an adenoviral genome having an E region deletion and three different, non-adenoviral restriction endonuclease sites located in the E region. The second vector is a shuttle vector and includes an insertion nucleic acid flanked by two of the three different non-adenoviral endonuclease sites present in the first vector. Cleavage products

Please replace Page 7, lines 3 to 29: with:

As indicated above, the first vector comprises an adenoviral genome. By adenoviral genome is meant the genome of an adenovirus, where adenoviral type 5 genomes are of particular interest. While in principle any type of vector may be employed, the first vector is typically a plasmid. A critical feature of the first vector employed in the subject methods is that the adenoviral genome of the first vector includes an E gene deletion. By E gene deletion is meant a deletion or absence of a portion of the adenoviral genome where the adenoviral E genes are present, e.g. E1, E2, E3, E4. The size of the E gene deletion of the subject first vector may vary, but in many embodiments typically ranges from about 1.0 to 35.0 kb, usually from about 1.0 to 12 kb and often from about 3 to 8 kb. In many embodiments, the E gene deletion includes a deletion of one of the E1, E3 and/or E4 genes, where in certain embodiments the deletion is characterized by a deletion of the E1 and E3 genes or a portion thereof, i.e. an E1/E3 deletion; and in other embodiments the deletion is a deletion of the E1 and E4 genes or a portion thereof, i.e. an E1/E4 deletion.

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The first vector is further characterized by the presence of first, second and third endonuclease restriction sites, where these sites are present in the E gene deletion region of the vector. The first, second and third restriction endonuclease sites are sites that are not found in the wild type adenoviral genome corresponding to the adenoviral genome present in the vector. For example, in those embodiments where the adenoviral genome is a type 5 adenoviral genome, the first, second and third restriction endonuclease sites present in the E deletion region of the adenoviral genome are ones that are not found in the wild type type 5 adenoviral genome. Furthermore, the first, second and third restriction endonuclease sites appear only once on the first vector. As such, they are unique on the first vector. In addition, the first, second and third restriction endonuclease sites are different from each other, i.e. they are recognized and cleaved by different restriction endonucleases. The order of the restriction endonuclease sites going in the direction from the E region to the remainder of the adenoviral genome is: first, second and third.

Please replace Page 9, lines 2 to 23 with:

invention). In other words, the non-adenoviral nucleic acid or insertion nucleic acid includes the nucleic acid which is desired to be inserted into the adenoviral genome of the first vector. Typically, the insertion sequence includes a multiple cloning site, i.e. a region having a plurality of different restriction endonuclease sites, where the number of different restriction endonuclease sites may vary greatly, but is often between about 1 to 20, usually between about 3 to 10. The insertion nucleic acid may or may not include a nucleic acid that encodes a product which is expressed under appropriate conditions. In other words, the insertion nucleic acid or non-adenoviral nucleic acid may or may not include a transgene. When present, this product encoding nucleic acid or gene is located in one of the restriction sites of the multiple cloning site of the insertion nucleic acid. The length of the insertion nucleic acid may vary, but in many embodiments typically ranges from about 1.0 to 35.0 kb, usually from about 1.0 to 12 kb and often from about 3 to 8 kb.

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Please replace Page 10, lines 19 to 27 with:

In many embodiments, the at least one transcriptionally active gene or module encodes a protein that has therapeutic activity for the multicellular organism, where such include genes encoding the following products: factor VIII, factor IX, β -globin, low-density protein receptor, adenosine deaminase, purine nucleoside phosphorylase, sphingomyelinase, glucocerebrosidase, cystic fibrosis transmembrane regulator, α -antitrypsin, CD-18, ornithine transcarbamylase, arginosuccinate synthetase, phenylalanine hydroxylase, branched-chain α -ketoacid dehydrogenase, fumarylacetoacetate hydrolase, glucose 6-phosphatase, α -L-fucosidase, β -glucuronidase, α -L-iduronidase, galactose 1-phosphate uridylyltransferase, interleukins, cytokines, small peptides etc, and the like.